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Quantification of food intake

Caroline Lei Wee Sam Kunes Erin Song

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An abbreviated version of this protocol was published in eLIFE in Oct 2019

A bidirectional network for appetite control in larval zebrafish

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Detailed protocol

Paramecia feeding assay with lipid dye

- **MATERIALS:**
  - DiD dye (Thermo Fisher D7757)
  - 4%PFA in PBS (PH7.2-7.4) stored at 4 degrees
  - Embryo water
  - Petri dishes
  - Paramecia cultures
  - Collection tube sieves and glass funnel

1. Prepare lipid dye in pure ethanol. For DiD dye, make10mg/ml stock solution and 2.5mg/ml working solution. Larval fish have autofluorescence that may interfere with imaging with lipid dyes at shorter wavelengths.
2. Rear fish under standard conditions in embryo water. Change water daily. Fish are fed excess unlabeled paramecia from 5dapf onwards daily as well as before experiments.
3. Harvest paramecia cultures through 25 uM sieve to remove algae. Rinse paramecia and use squeeze bottle to wash off into a beaker. Transfer collected paramecia into 50ml Falcon tubes. Spin down gently (<3000 rpm) at room temperature.
4. Re-suspend pellet in 1ml deionized water and aliquot 200ul each into 1.5 ml Eppendorf tubes. Add more deionized water to a total volume of 800ul. Add 5ul of 2.5mg/ml lipid dye to each tube. Cover tubes with foil and gently shake them on the side on a rotator at room temperature for 2 hours.
5. Centrifuge labeled paramecia at <3000rpm and remove supernatant. Re-suspend in 1ml deionized water and centrifuge to wash away excess ethanol and dye. Re-suspend and combine paramecia from all tubes in 1 ml deionized water or embryo water. Calculate how much paramecia are needed for experiments and expand the volume accordingly. For more detailed paramecia quantification, please see Jordi et al. 2015, A high-throughput assay for quantifying appetite and digestive dynamics.
6. Prepare fish for experiments by removing unlabeled paramecia. Use plastic pipette to transfer fish into a new dish. Remove water carefully with pipetting, and add fresh fish water, repeat a few times to remove paramecia thoroughly. A quicker method to remove paramecia is to transfer fish into a chamber with a mesh at the bottom, so that paramecia all pass through the mesh. Fish are then rinsed and transferred to individual petri dishes.
7. An equal amount (100 µl, ~500 paramecia) are pipetted into each 10 cm dish with 10-15 larval fish. Add more paramecia for larger groups of fish or longer feeding duration.
8. After the feeding experiment, transfer larval fish quickly through a glass funnel into a tubed sieve (tube sieves are prepared from 15ml falcon tubes. Heat the opening of a tube with Bunsen burner, then press firmly against a plastic mesh and cut the tube in half). Drop the tube sieve into a 50ml falcon tube with 5ml of 4%PFA. Fix larvae in 4% PFA for 1 hour or overnight at 4 degrees.
9. Transfer fish out into glass tubes or Eppendorf tubes, rinse once quickly with 1XPBS (do not use PBT), and transfer and place larvae on their side in wells of a flat-bottom 96 well plate, either individually or in a group of 4 or 5 (avoid having guts of these fish touch each other) and remove excess PBS (do not let fish dry out, leave a small amount of PBS around individual fish). Other flat bottom dishes can be used instead of a 96 well plate for imaging purposes.
10. Image 4 wells at a time using the AxioZoom V16 (Zeiss) and analyze using custom Fiji (Schindelin et al., 2012) software. To image without fixation, please see Jordi et al., 2015.

1. Wee, C. , Kunes, S. and Song, E. (2021). Quantification of food intake. Bio-protocol Preprint. bio-protocol.org/prep1116.
2. Wee, C. L., Song, E. Y., Johnson, R. E., Ailani, D., Randlett, O., Kim, J., Nikitchenko, M., Bahl, A., Yang, C., Ahrens, M. B., Kawakami, K., Engert, F. and Kunes, S.(2019). A bidirectional network for appetite control in larval zebrafish. eLIFE. DOI: 10.7554/eLife.43775